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Detection of *Yersinia enterocolitica* in raw pork by conventional culture methods and PCR based methods

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Abstract

Yersinia enterocolitica is an emerging pathogenic microorganism associated with food. Its ingestion, through contaminated food, may cause different kinds of intestinal disorders. Since there is not much information about the presence of *Y. enterocolitica* concerning the consumption of food in Portugal and the conventional methodology is not very effective, this study proceeded, by implementing the PCR methodology, in order to detect the pathogenic microorganism in pork meat.
One hundred samples of raw minced meat were acquired in supermarkets and butcher's shops in the Greater Oporto and Braga area, with the purpose of determining the occurrence of *Y. enterocolitica*.
The detection limit of the conventional method (ISO 10273: 2003) was determined to be 10^5 CFU/g using CIN culture medium and 10^4 CFU/g using a pre-treatment step with KOH, which highlights the difficulties in detecting *Yersinia* using this methodology. A molecular PCR-based method was implemented, using BDC followed by cellular alkaline lysis to extract the samples' DNA (BDC-PCR-based method). The primers used in this study were the 16S rRNA gene, which allowed the detection of the genera *Yersinia,* and the *yst* gene to detect the pathogenic strains of the microorganism. The detection limit was studied in both sets of primers. The values obtained were 10^2 CFU/g for the 16S rRNA gene and 10^3 CFU/g for the *yst* gene for a pre-enrichment time of 24 h. Nevertheless, we have implemented a combined culture and PCR method for detection of *Yersinia* that besides ensuring the viability of the cells detected, showed to be more sensitive than the BDC-PCR-based method. All the samples were analysed by the BDC-PCR-based method and 25 by the three methodologies. The different methodologies will be discussed and the incidence of *Y. enterocolitica* in raw minced meat pork evaluated.
The results of this study show that the molecular methodology and the combined methodology that were here adopted are more sensitive than the common methodology. Therefore, it can become an important tool in food sample control, since it allows quicker results in a smallest time span. It is also more reliable and easier to work with when compared to other conventional methods.